Supplementary data

Supplementary table 1. Full inclusion and exclusion criteria

Inclusion criteria
1. Male 2. Age 18 years or older 3. Subjects with congenital hemophilia B classified as one of the following: • Known severe Factor IX (FIX) deficiency (< 1% of normal circulating FIX) for which the subject is either on continuous routine FIX prophylaxis* or using on-demand FIX replacement therapy. • Known moderately severe FIX deficiency (1-2% of normal circulating FIX, inclusive) and a severe bleeding phenotype as defined by at least one of the following: i. On continuous routine FIX prophylaxis* for a history of bleeding ii. On demand FIX replacement therapy with a history of frequent bleeding (4 or more bleeding episodes in the last 12 months) or chronic hemophilic arthropathy (pain, joint destruction, and loss of range of motion) in one or more joints 4. >20 previous exposure days of treatment with FIX protein 5. Acceptance to use a condom during sexual intercourse in the period from etranacogene dezaparvovec administration until AAV5 has been cleared from semen, as evidenced by the central laboratory from negative analysis results for at least three consecutively collected semen samples (this criterion is applicable also for subjects who are surgically sterilized) 6. Able to provide informed consent following receipt of verbal and written information about the trial. **Continuous routine prophylaxis is defined as the intent of treating with an a priori defined frequency of infusions (e.g. twice weekly, once every two weeks, etc.) as documented in the medical records*
Exclusion Criteria:
1. History of FIX inhibitors 2. Positive FIX inhibitor test at screening 3. Screening laboratory values: • ALT >2 times upper normal limit • AST >2 times upper normal limit • Total bilirubin >2 times upper normal limit • Alkaline phosphatase (ALP) >2 times upper normal limit • Creatinine >2 times upper normal limit 4. Positive human immunodeficiency virus (HIV) serological test at screening, not controlled with anti-viral therapy as shown by CD4+ counts = 200/µL or by a viral load of > 200 copies/mL 5. Active infection with hepatitis B or C virus as reflected by hepatitis B surface antigen (HBsAg), hepatitis B extracellular antigen (HBeAg), hepatitis B virus deoxyribonucleic acid (HBV DNA) or hepatitis C virus ribonucleic acid (HCV RNA) positivity, respectively, at screening 6. History of hepatitis B or C exposure, currently controlled by antiviral therapy 7. Known coagulation disorder other than hemophilia B 8. Thrombocytopenia, defined as a platelet count below 50×10^9/L, at screening 9. Known severe infection or any other significant concurrent, uncontrolled medical condition including, but not limited to, renal, hepatic, cardiovascular, hematological, gastrointestinal, endocrine, pulmonary, neurological, cerebral or psychiatric disease, alcoholism, drug dependency

or any other psychological disorder evaluated by the investigator to interfere with adherence to the protocol procedures or with the degree of tolerance to the IMP

10. Known significant medical condition that may significantly impact the intended transduction of the vector and/or expression and activity of the protein, such as disseminated intravascular coagulation, accelerated fibrinolysis, and profound liver fibrosis
11. Known history of an allergic reaction or anaphylaxis to FIX products
12. Known uncontrolled allergic conditions or allergy/hypersensitivity to any component of the IMP excipients
13. Known medical condition that would require chronic administration of steroids
14. Previous gene therapy treatment
15. Receipt of an experimental agent within 60 days prior to screening
16. Current participation or anticipated participation within one year after IMP administration in this trial in any other interventional clinical trial involving drugs or devices

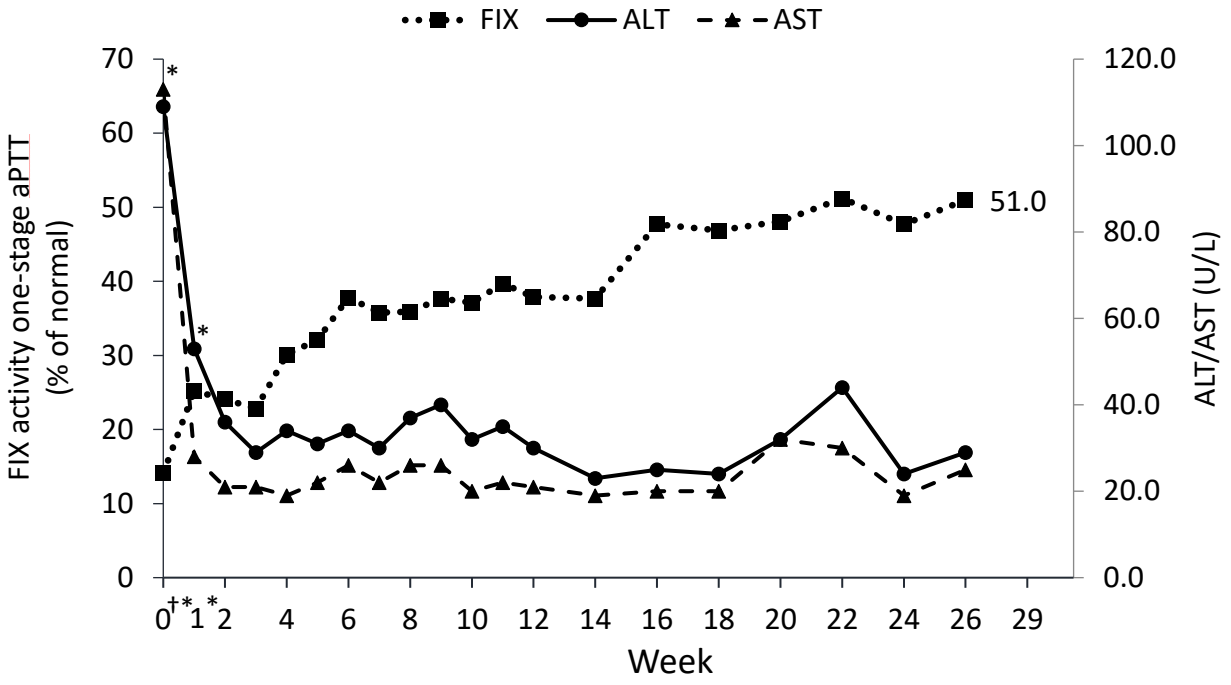
Supplementary Table 2. Inflammatory markers

Week	Participant 1					Participant 2					Participant 3				
	IFNγ	IL-1β	IL-2	IL-6	MCP-1	IFNγ	IL1β	IL2	IL6	MCP-1	IFNγ	IL-1β	IL-2	IL-6	MCP-1
0	3.39	<LOD	<LOD	<LOD	815.6	<LOD	<LOD	<LOD	<LOD	394.9	3.44	<LOD	<LOD	<LOD	476.1
1	13.48	<LOD	<LOD	1.09	759.4	28.11	<LOD	<LOD	<LOD	493.1	4.56	<LOD	<LOD	<LOD	363.4
2	4.56	<LOD	<LOD	<LOD	542.0	7.77	<LOD	<LOD	<LOD	460.5	4.20	<LOD	<LOD	<LOD	460.5
3	2.93	<LOD	<LOD	<LOD	530.9	8.88	<LOD	<LOD	<LOD	560.4	4.27	<LOD	<LOD	<LOD	434.3
4	<LOD	<LOD	<LOD	<LOD	608.8	5.18	<LOD	<LOD	<LOD	462.0	<LOD	<LOD	<LOD	<LOD	524.9
5	<LOD	<LOD	<LOD	<LOD	625.9	4.23	<LOD	<LOD	<LOD	409.5	<LOD	<LOD	<LOD	<LOD	466.0
6	<LOD	<LOD	<LOD	<LOD	571.3	3.61	<LOD	<LOD	<LOD	399.0	<LOD	<LOD	<LOD	<LOD	389.3
7	<LOD	<LOD	<LOD	<LOD	642.9	4.73	<LOD	<LOD	<LOD	387.7	<LOD	<LOD	<LOD	1.12	477.8
8	<LOD	<LOD	<LOD	<LOD	747.5	3.39	<LOD	<LOD	<LOD	375.5	<LOD	<LOD	<LOD	<LOD	408.9
9	<LOD	<LOD	<LOD	<LOD	701.3	5.36	<LOD	<LOD	<LOD	393.2	3.25	<LOD	<LOD	<LOD	403.9
10	<LOD	<LOD	<LOD	<LOD	611.6	3.82	<LOD	<LOD	<LOD	412.2	13.12	<LOD	<LOD	1.19	448.2
11	<LOD	<LOD	<LOD	<LOD	561.4	3.28	<LOD	<LOD	<LOD	360.5	<LOD	<LOD	<LOD	<LOD	454.1
12	<LOD	<LOD	<LOD	<LOD	639.3	3.63	<LOD	<LOD	<LOD	332.4	<LOD	<LOD	<LOD	<LOD	411.4
14	<LOD	<LOD	<LOD	<LOD	341.2	7.38	<LOD	<LOD	<LOD	358.1	4.55	<LOD	<LOD	<LOD	494.4
16	<LOD	<LOD	<LOD	<LOD	594.6	10.68	<LOD	<LOD	<LOD	397.4	NT	NT	NT	NT	NT
18	<LOD	<LOD	<LOD	<LOD	607.8	<LOD	<LOD	<LOD	<LOD	326.4	6.99	<LOD	<LOD	1.05	563.3
20	<LOD	<LOD	<LOD	<LOD	689.0	<LOD	<LOD	<LOD	<LOD	329.0	3.76	<LOD	<LOD	<LOD	515.3
22	<LOD	<LOD	<LOD	<LOD	597.9	<LOD	<LOD	<LOD	<LOD	341.9	3.25	<LOD	<LOD	1.51	454.5
24	<LOD	<LOD	<LOD	<LOD	442.8	<LOD	<LOD	<LOD	<LOD	353.8	2.94	<LOD	<LOD	<LOD	400.7
26	<LOD	<LOD	<LOD	<LOD	549.5	4.12	<LOD	<LOD	2.47	245.4	3.48	<LOD	<LOD	<LOD	487.6

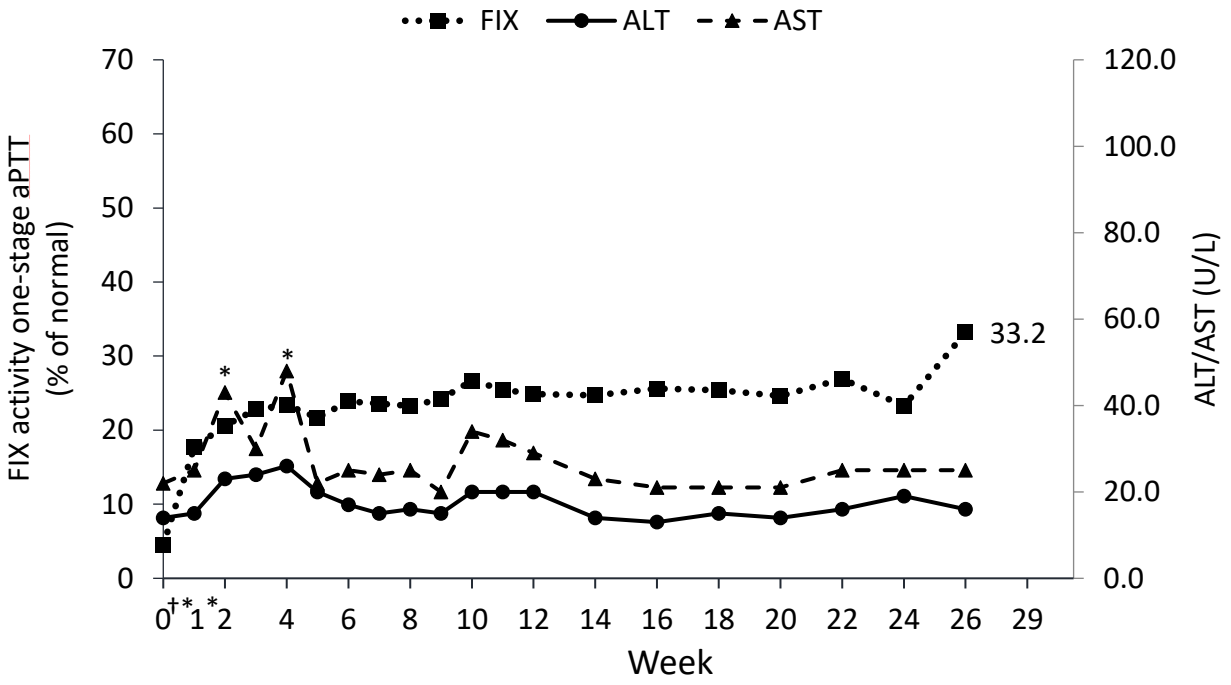
Results are pg/mL. Flagged high values are indicated by grey shading. Reference ranges-IFNγ: 2.86-2920[a]/23360[b], where [a] is x2 dilution and [b] is extended Analytical Measurement Range (AMR); IL-1β <0.61; IL-2 the ref range is 0.72-2920[a]/23360[b], where [a] is x2 dilution and [b] is extended AMR; IL-6 <8.60; MCP-1 200-722. IFNγ, interferon gamma; IL-1, interleukin-1; IL-6, interleukin-6; LOD, limit of detection; MCP-1, monocyte chemoattractant protein-1; ND, not tested.

Supplementary figure 1. FIX activity, ALT and AST levels over time

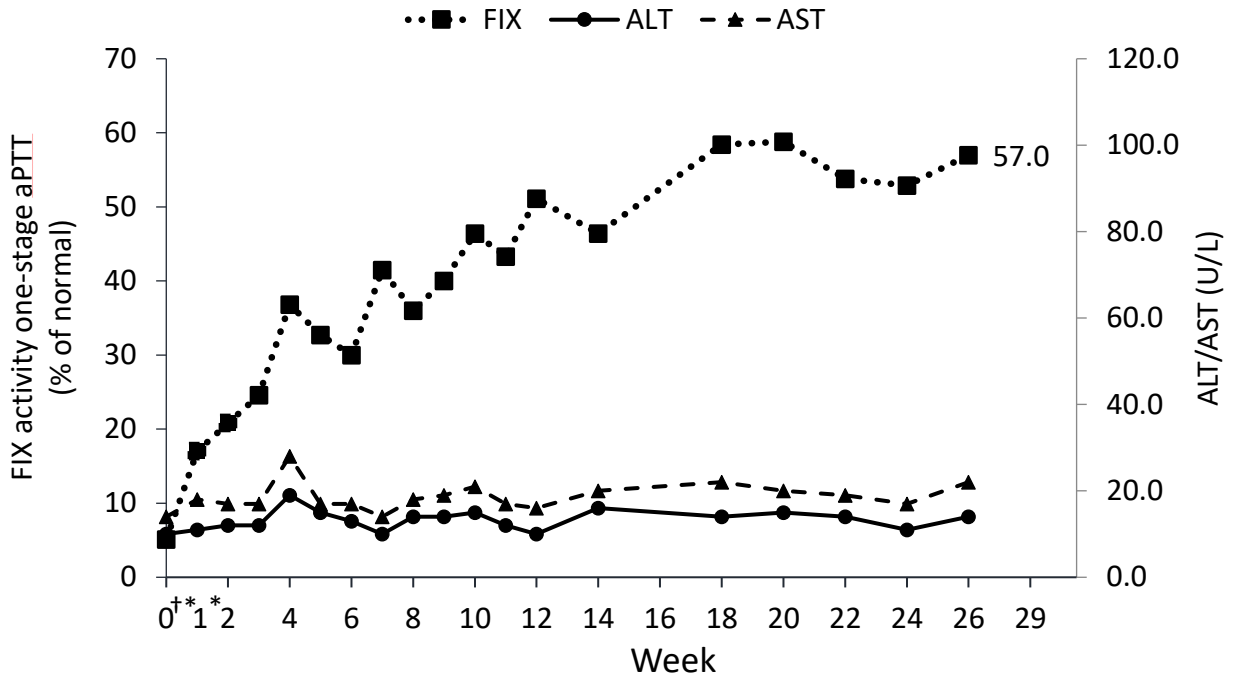
a. Participant 1



b. Participant 2



c. Participant 3



†The Week 0 time point reflects FIX activity before etranacogene dezaparvovec treatment. Participant 1 had normal transaminase levels at screening for study eligibility, but later analysis of the baseline samples indicated that ALT (109 U/L, reference range 6-41 U/L) and AST (113 U/L, reference range 9-34 U/L) levels were elevated prior to the gene-transfer procedure on the day of etranacogene dezaparvovec dosing. *Samples may include activity from exogenous FIX replacement. ALT reference range 6-41 U/L. AST reference range 9-34 U/L. *Denotes values above the reference range. ALT, alanine aminotransferase; aPTT, activated partial thromboplastin time; AST, aspartate aminotransferase; FIX, Factor IX.